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The effect of exogenous glycine betaine on yield of soybean [*Glycine max* (L.) Merr.] in two contrasting cultivars Pershing and DPX under soil salinity stress

Mohammad Ali Rezaei[1*], Behzad Kaviani[2] and Ardashir Kharabian Masouleh[3,2]

[1]Department of Biology, Gorgan Branch, Islamic Azad University, Gorgan, Iran
[2]Department of Horticultural Science, Rasht Branch, Islamic Azad University, Rasht, Iran
[3]Southern Cross Plant Science, Southern Cross University, NSW 2480, Australia

***Corresponding author: mohalirezaei@yahoo.com**

Abstract

Salinity stress restricts growth of soybean plant [*Glycine max* (L.) Merr.]. Glycine betaine (GB) is among osmoprotectant compounds that are produced in tolerant plant species in response to environmental stresses. Soybean is sensitive to soil salinity and is classified as a low-ccumulator of glycine betaine. Therefore, this study was performed to evaluate the salinity tolerance of two contrasting soybean [*Glycine max* (L.) Merr.] cvs. Pershing and DPX at field saline soils (EC=11.1 dS/m^{-1}). The exogenous glycine betaine (Exo-GB) treatments (0, 2.5, 5, 7.5 and 10 kg/ha) were applied in six foliar and near the flowering stages. During the growth period the amount of endogenous-glycine betaine (Endo-GB) was measured in ten foliar stages and in leaves of seeding stage. Results showed that the uptake of Na^+ decreased in response to increment of Exo-GB levels, in which tolerant cv. DPX (24%) had a greater capacity to prevent Na^+ uptake. Endo-GB had higher concentrations in younger leaves than in mature leaves. Exo-GB increased the number of lateral branches significantly (33%) and especially pods per plants (49%) in cv. DPX. There was no difference in seed number per pod between controls and all levels of Exo-GB treatments in two cultivars. All treatments of Exo-GB significantly increased weight of thousands grain (highest; 71% in 10 kg ha^{-1} GB) in salt tolerant cv. DPX. Application of Exo-GB on weight of thousands grain was cultivar-, dose-, and time-dependent. The grain yield of soybean was increased by foliar applications of Exo-GB. This was due to significant increase in number of lateral branches and pods and weight of thousands grain, without significant different between cultivars.

Keywords: Salinity stress, soybean, exogenous glycine betaine, yield.
Abbreviations: GB_Glycine Betaine; Exo-GB_Exogenous Glycine Betaine; Endo-GB_Endogenous Glycine Betaine.

Introduction

Plants tend to be exposed to many stress factors, such as drought, high salinity or pathogens, which reduce the yield of the cultivated plants or affect the quality of the harvested products (Arafa et al., 2009). Salinity, in particular, is the most widespread problem, affecting approximately 20% of the world's cultivated land and nearly half of the area under irrigation (Flowers and Yeo, 1995). Salt stress can directly or indirectly affect the physiological status of plants by disturbing their metabolism, growth, development and productivity (Zhu, 2001) and is known to affect many aspects of metabolism, anatomy and ultra-structure of plant cells (Rahman et al., 2000). Salinity causes a number of changes in plant metabolism, through ion toxicity and osmotic stress (Mittler, 2002). Soybean is classified as a moderately salt-tolerant crop and the final yield of soybean will be reduced when soil salinity exceeds 5 dS/m^{-1} (Ashraf, 1994). It was shown that the average production of 20 soybean cultivars under non-saline conditions, 14-15 dS/m^{-1}, and 18-20 dS/m^{-1} were 2261.4±438.3 kg/hm^2 (control), 1073.4±267.1 kg/hm^2 (47.5% of control), and 880.8±259.9 kg/hm^2 (38.9% of control), respectively (Chang et al., 1994). Under salt stress, some plants, such as halo-tolerant plants, accumulate a compatible osmolyte-glycine betaine (Grieve and Maas, 1984; Hanson and Wyse, 1982; Park et al., 1995). The accumulated GB may maintain cellular osmotic balance (McCue and Hanson, 1992) and stabilize quaternary structures of complex proteins (Papageorgiou and Murata, 1995). GB may also protect membrane functions from high concentrations of Na^+ and Cl^- (Rhodes and Hanson, 1993). Uptake of foliar applied GB has been shown to be active (Ladyman et al., 1980). GB was absorbed by the leaves and remained stable there, indicating a long-term protective capability. Exo-GB also improves the growth, survival, and tolerance of a wide variety of accumulator/non-accumulator plants under various stress conditions (Harinasut et al., 1996; Rajasekaran et al., 1997; Diaz-Zorita et al., 2001). GB effects were highly significant when it was applied at a critical growth stage (Agboma et al., 1997). Levels of GB accumulation are correlated with the extent of salt tolerance by plants (Rhodes and Hanson, 1993). Exogenous application of GB to plants, that accumulates little or none of this compound, may help to reduce the adverse effects of environmental stress (Yang and Lu, 2005; Ashraf and Foolad, 2007). There are many reports demonstrating the positive effects of Exo-GB on plant growth and final crop yield under drought stress. Examples include those in tobacco, wheat, barley, sorghum, soybean and common beans (*Phaseolus vulgaris*)(Ashraf and Follad, 2007). There is evidence that soybean could be classified as a low-accumulator of GB (Agboma et al., 1997). More importantly, Exo-GB application also increases the salt tolerance of some plants that are not able to accumulate GB (Harinasut et al., 1996; Hayashi et al., 1998; Makela et al., 1999). There is a positive correlation between the level of Endo-GB and the degree of salt tolerance in plants (Meek and Oosterhuis, 2000; Naidu et al., 1998). The aim of this study was; (1) to evaluate the effects of exogenously applied GB in vegetative and near the reproductive stages of soybean [*Glycine max* (L.) Merr.] cv. Pershing and DPX under soil saline stress, (2) to determine endogenous levels of GB in response to salinity stress, (3) to characterize long-term responses between tolerant and sensitive cultivars treated with GB and, (4) to evaluate the potential use of GB to enhance yield and salinity tolerance in soybean.

Results and Discussion

Effects of GB on Na^+ uptake

GB treatments affected the amounts of Na^+ uptake in both soybean cultivars (Fig 1). Differences in amounts of Na^+ uptake were significant between both cultivars. However, amount of Na^+ (or uptake by root or accumulation in leaves) decreased in response to increasing Exo-GB levels. This decrease was more significant in cv. DPX (34, 21, 19, 19 and 20 mg/g DW) than in cv. Pershing (43, 42, 40, 33 and 33 mg/g DW). There was no significant difference in Na^+ uptake at all Exo-GB applications, except for control of cv. DPX (Fig 1). *Glycine max* cv. DPX possessed higher salt tolerance than *G. max* cv. Pershing. Tolerant genotypes had a greater capacity to prevent Na^+ transport from soil solution to leaves than sensitive genotypes. Magnitude of leaf injury per unit increased in Na^+ concentrations was lower in leaves of tolerant than susceptible accessions (Lenis, 2010). The major differences in Na^+ transport between the genotypes were; (1) the rate of transfer from the root to the shoot (xylem loading), which was much lower in the salt tolerant genotype (as cv. DPX), and (2) the capacity of the leaf to extract Na^+ as it entered the leaf (Romola et al., 2005). It was also observed that GB-treated plants under salinity stress had significantly lower Na^+ and higher K^+ concentrations in shoots, compared to untreated plants. Hence, GB may also help in salinity tolerance through its role in signal transduction and ion homeostasis (Rohman et al., 2002).

Effects of GB on $C1^-$ uptake

The Cl^- distribution in the plants showed an opposite pattern to that of Na^+ (Fig 2). This distribution pattern was previously observed in soybean (Durand, 1994; Abel et al., 1964; Velagaleti et al., 1992). The Cl^- accumulation in cv. DPX was higher than that of cv. Pershing in the control plants, suggesting a higher Cl^- requirement under normal conditions. With increasing Exo-GB level, the Cl^- accumulation in leaves increased by Exo-GB application of 7.5 and 10 kg $ha^{-1}$of in cv. Pershing and only in 7.5 kg ha^{-1} in cv. DPX. The Cl^- accumulation in Exo-GB levels contradicts with previous reports that shoed salt tolerant cultivars accumulate less Cl^- than salt sensitive ones (Velagaleti et al., 1992). However, Pantalone et al. (1997) suggested that some salt tolerant plants used the uptake of Cl^- for osmotic adjustments. The concentration at which Cl^- shows a harmful effect on plant growth should be further studied.

Effects of Exo-GB treatments on Endo-GB content

Endo-GB measurements in ten foliar plant showed that Exo-GB treated plants had higher levels of GB and there was no significant difference between GB contents in all treatments, except for control, in both cultivars (Fig 3). There is evidence that soybean is normally a low-accumulator of GB (Agboma et al., 1997). Foliar application of GB could increase its content in soybean plant, leading to an improvement in photosynthesis activity, nitrogen fixation, leaf area development, and seed yield of both well-irrigated and drought-stressed soybean plants (Makela et al., 1996; Agboma et al., 1997). Therefore, foliar application of GB is a potential strategy to enhance salt tolerance in soybean. In fact, it has been shown that exogenous GB application can increase the salt tolerance of plants that are not able to accumulate GB (Harinasut et al., 1996; Hayashi et al., 1998; Makela et al., 1999). Some maize genotypes are not able to synthesize GB (Rhodes et al., 1989) and some others genotypes can accumulate it. The accumulation of GB in some stressed maize plants is about 10-fold lower than those in stress-tolerant plants (Allard et al., 1998; Grieve and Maas, 1984; Kishitani et al., 1994). The GB levels in stressed plants were increased by 31.1 and 68% during the vegetative and reproductive stages, respectively (Ashraf, 1994; Foolad, 1999b; Foolad and Lin, 2001). Endo-GB content may vary among genotypes, and a positive correlation exists between the level of Endo-GB and final crop yield in soybean (Ashraf and Follad, 2007). Exo-GB treatments in reproductive (or seed) stage lead to similar GB accumulation in comparison with control (Fig 4). The GB accumulation was greater in younger leaves (plant of ten foliar) than mature and old leaves compared to former vegetative stage (Fig 3 and Fig 4), while induction of GB increased by salt stress was greater in mature and old leaves than in younger leaves (Wang et al., 2004). In this study, we confirmed that GB levels in soybean leaves have not changed at flowering stage when plants are exposed to soil saline stress, even though after exogenous application of GB before the stages of flowering (Fig 4). Our report describes variation in GB content at different stages by developmental status of soybean. It is assumed that the age, at which plants are exposed to Exo-GB, plays a critical role (Chen et al., 2009). Several papers have described GB accumulation in the mature leaves of *Amaranthus hypochondriacus* L. (Legaria et al., 1998), *A. caudatus* L. (Russell et al., 1998), and *A. tricolor* (Wang et al., 1999). Betaine is synthesized continuously during growth and its synthesis probably occurs in the mature leaves (Hanson and Nelsen, 1978; Hanson and Scott, 1980). Both exogenously supplied [14C] betaine, and betaine endogenously synthesized from [14C] betaine aldehyde are readily moved from the second leaves, apparently in the phloem (Juanita et al., 1980). However, in the maturation stage a decrease of 18.5 to 9 mg/g DM was recorded after the stress simulation. The maximum values in control and stress plants were found during the reproductive stage (Oliveira et al., 2009). The GB reduction during the maturation stage is linked to leaf senescence and consequent chloroplast degradation. The maximum value in reproductive stage is probably due to the amino acids formation through the nitrogen metabolism in control treatment and protein breakdown in stress treatment (Oliveira et al., 2009). Some preliminary reports on cereals indicate that once synthesized, betaine may not be further metabolized and may be mobile within the plant (Ahmad and Wyn Jones, 1979; Bowman and Rohringer, 1970; Hanson et al., 1978). In barley, after relief of stress, the betaine is

Table 1. Number of latral branches per plant, pods per plant, grain per pods and weight of 1000 grain in soybean [*Glycine max* (L.) Merr.] cv. Pershing and DPX in saline soil stress after exogenous application of GB. Values represent mean-SE of four replications plants.

Exo-GB treatment (Kg/ha)	Cultivar	Number of lateral branches/plant	Number of pods/plant	Number of grain/pod	Weight of 1000 grain (g)
0	DPX	3.50±0.27e	38±5.70f	1.80±0.13a	130.33±3.00e
	Pershing	3.31±0.25e	38±1.70f	1.68±0.12a	123.50±8.80e
2.5	DPX	4.31±0.21cd	55±4.20bcd	1.87±0.09a	198.80±9.11bc
	Pershing	4.00±0.42de	49±3.40bcde	1.54±0.12a	132.80±9.70e
5	DPX	4.81±0.21bcd	69±4.37ab	1.57±0.10a	216.20±3.00ab
	Pershing	5.37±2.3ab	61±4.10abc	1.71±0.11a	132.50±8.21e
7.5	DPX	5.06±0.21ab	73±1.35ab	1.59±0.14a	187.60±3.85c
	Pershing	6.31±0.44a	62±5.50abc	1.68±0.04a	125.10±6.65e
10	DPX	5.24±0.17ab	75±4.80a	1.63±0.08a	223.80±11.0a
	Pershing	5.00±0.28ab	60±2.10abc	1.66±0.06a	142.60±3.09d

exported from the expanded leaves to the younger ones, actively growing regions of the shoot (Juanita et al., 1980). The translocation of GB takes effect immediately after application and GB is translocated easily to the developing organs (Yang and Lu, 2005). GB is an inactive molecule in plant cells and can be translocated via phloem (Makela et al., 1996). The stability of GB, in terms of practical utility in crop production, is sufficient and GB remains un-metabolized up to 17 days after application (Makela et al., 1996). When [14C] GB solution was exogenously applied to the foliage of summer turnip rape (*Brassica rapa* L. ssp. oleifera), [14C] GB was translocated to roots within two hours of application. One day after application, labeled GB was translocated to all plant parts of turnip rape plants and plants were able to translocate foliar-applied GB from their leaves to other organs, indicating that the use of surfactants accelerates the penetration of foliar-applied GB (Makela et al., 1996). According to our results, GB is quite inert end-product in plant cells being mainly phloem-mobile. Moreover, environmental conditions are shown to affect the uptake and translocation rates of foliar-applied GB (Makela et al., 1996). There are many reports demonstrating the positive effects of exogenous application of GB on plant growth and final crop yield in soybean (Ashraf and Follad, 2007). There are a few reports suggesting a lack of such positive effects or even apparent negative effects of Exo-GB on plants growing under stress conditions. Foliar application of GB did not affect yield components or endogenous levels of GB in cotton plants grown under drought stress (Meek et al., 2003). It has also been reported that plants are able to utilize foliar-applied GB and to translocate it to almost all plant parts, especially developing organs (Makela et al., 1996b). Variation of their levels among different organs and at different ages were examined and GB content was markedly different among cotyledons, between roots, stems, leaves, and flowers (including seeds) (Wang et al., 2004). The GB contents of these organs were very low during the earlier stages of plant development and increased as the plant developed. Roots accumulated a small amount of GB at all stage of plant development. Salt stress induced an accumulation of GB in most organs, except cotyledons and roots. The GB content within the cotyledon was changed as a result of salt stress. The GB content was higher in leaves just before and after they unfolded and was lower in mature and older leaves. Salt stress triggered a marked induction of the production of GB in mature and old leaves (Wang et al., 2004). Flowers (without seeds) contained GB, while the developing seeds had lower GB contents. Salt stress did not induce production of GB in seeds, but induced its production in flowers. Thus, GB in the seeds must be translocated from maternal plants during the seed development. It is suggested that the GB remains in dried seeds after it is imported from flowers and plants (Wang et al., 2004). Foliar applied GB is readily taken up and translocated within hours to developing leaves, probably along with assimilation products (Mackela et al., 1996a). However, the rate of uptake and consequent GB concentration in the plant tissue seem to be not only dependent on plant organ and its age but also on crop species and environmental factors (Mackela et al., 1996a). Co-application of surfactant and vegetable oils in particular, tends to increase the GB uptake probably due to faster uptake rate and minimizing the risk of rain washing off of GB from the leaf foliage.

Effects of GB on the number of lateral branches, pods and number of seed per pod

Exo-GB application increased significantly the number of lateral branches and pods per plant in saline soil stress in both soybean cultivars (Table 1). This increase was higher in cv. DPX. The agronomic traits of soybean could severely be affected by high salinity, including reduction in height, leaf size, biomass, and number of internodes, number of branches, number of pods, weight per plant, and weight of 100 seeds (Abel and MacKenzie, 1964; Chang et al., 1994). No difference was observed in seed number per pod between controls and all treatments of Exo-GB applications in cv. Pershing and DPX in saline soil stress (Table 1). Reports in soybean showed increase in number of seeds, dry weight of pods, weight of total and large seeds, following the application of 3kg ha^{-1} GB (Agboma, 1997). In comparison with control, all treatments of Exo-GB application increased weight of 1000 grains in saline soil stress in cv. DPX, significantly (Table 1). This increase was higher in cv. DPX. There was no difference between control and Exo-GB treatments in cv. Pershing, except for 10 kg ha^{-1} of GB that increased significantly (Table 1). Agboma et al. (1997) reported that the application of GB in the 50% irrigation regime on soybean reduced weights of seeds and in the 75 and 100% irrigation levels showed increases in weight of total and large seeds. The seed yield increase following the application of 3 kg ha^{-1} GB could be associated with the greater number of filled seeds and more large seeds (Agboma et al., 1997). However, Bergmann and Eckert (1984) reported that the application of GB to winter wheat enhanced grain yield that was related to a larger number of grains per plant. Exogenous application of GB either in the form of foliar spray or seed treatment in sunflower had no effect on

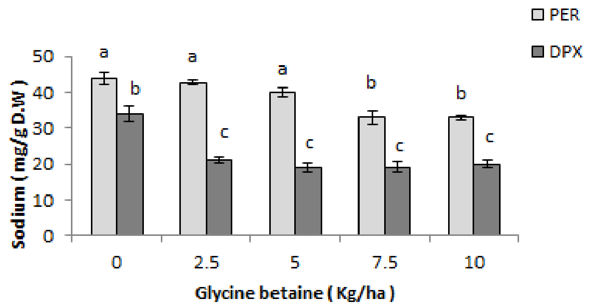

Fig 1. Leaf sodium contents in soybean [*Glycine max* (L.) Merr.] cv. Pershing and DPX in saline soil stress after exogenous application of GB. Values represent mean-SE of four replications plants.

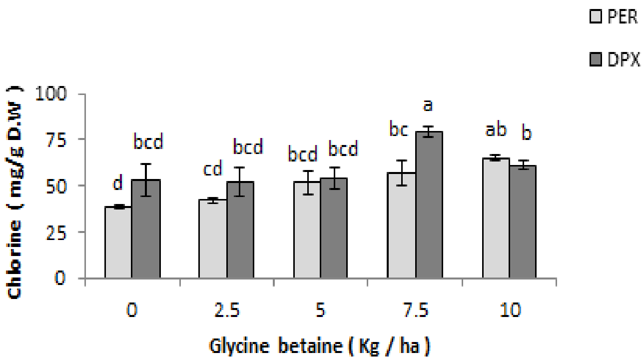

Fig 2. Leaf chloride contents in soybean [*Glycine max* (L.) Merr.] cv. Pershing and DPX in saline soil stress after exogenous application of GB. Values represent mean-SE of four replications plants.

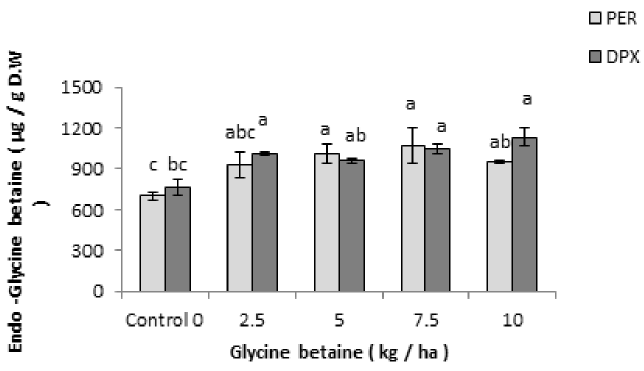

Fig 3. GB content in the leaves of soybean [*Glycine max* (L.) Merr.] cv. Pershing and DPX in ten-foliar stage in saline soil stress after exogenous application of GB. Values represent mean-SE of four replications plants.

weight of hundred achene under normally irrigated conditions. When the water deficit was imposed at the vegetative stage, exogenous application of GB was much more effective (Iqbal et al., 2005) and when water deficit was imposed at the reproductive stage, hundred achene weight was significantly higher (Iqbal et al., 2005). The effect of pre-sowing GB treatment was not prominent in terms of 100 seed weight (Tahir et al., 2009). Therefore, application of Exo-GB on soybean weight of 1000 grain in stress is time-, cultivar- and dose-dependent. Iqbal et al. (2005) reported that foliar application of GB at vegetative stage is more beneficial in alleviating the effects of water stress and improving the 100 achene weight and thus increasing achene yield of sunflower.

Effects of GB on grain yield per plant

On the basis of our research, field experiments conducted in saline soil stress have indicated that the grain yield of soybean cv. Pershing and DPX were significantly increased due to foliar applications of Exo-GB, when sprayed during the stress condition (Fig 5). The increase of seed yield following the application of Exo-GB is associated with the greater number of lateral branch and pod number per plant in both cultivars and more weight of 1000 grain (larger seeds) in cv. DPX (Table 1). It was reported that increase of seed yield by application of 3 kg ha^{-1} GB could be associated with the greater number of filled and larger sized seeds (Agboma, 1997).

Field experiments have indicated that the fruit yield of tomato plants increased by 29-39% under heat and salt stress when GB was applied during the mid flowering stage. Similar trends have been obtained with approximate increase of 20-30% in experiments conducted in the greenhouse of commercial vegetable grower in Finland but with unstressed tomato plants (Makela et al., 1998a). These results were reported for soybean [*Glycine max* (L.) Merr.], which Exo-GB tended to increase leaf area and seed yield (Agboma et al., 1997c). There are many reports demonstrating positive effects of exogenous application of GB on plant growth and final crop yield under drought stress. Examples include those in tobacco, wheat, barley, sorghum, soybean (Ashraf and Foolad, 2007). Agboma et al. 1997 applied the GB at pod initiation stage in the 75% watering regime. Their results showed that application of 3 kg ha^{-1} GB increases the grain yield that was 22% greater compared to 1 kg ha^{-1} GB treatment. Agboma et al. (1997a) concluded that the application of GB improved drought tolerance and increased the yield of maize and sorghum on field conditions. They did not observe any drought response in wheat under same condition. They also found that exogenous GB delayed canopy senescence of barley, oat and wheat, but these differences were not associated with differences in grain yields (Makela et al., 1996a). Foliar application of GB did not affect yield components or endogenous levels of GB in cotton plants grown under drought stress (Meek et al., 2003). There has also been indications that GB treatment induces grain yield increase, up to 25%, in maize (*Zea mays* L.), and up to 11% in sorghum [*Sorghum bicolor* (L.) Moench] when plants are suffering drought. The explanation of yield increase of stressed-plants after application of GB has been proposed to be partly located on the increased net photosynthesis, decreased rate of photorespiration, stomatal conductance, induced more efficient gas exchange (Makela et al., 1998b) and thus, better availability of carbon for photosynthetic processes and ability to avoid possibly photo-inhibition (Makela et al., 1999), water use efficiency (Bergmann and Eckert, 1984), chloroplast ultra-structure (Makela et al., 2000), chloroplast volume (Rajasekaran et al., 1997) and increased chlorophyll content in plants (Whapman et al. 1993; Blunden et al., 1997). In addition, GB protects, stabilizes and activates the proteins of photosynthetic reactions (Papageourgiou and Murata, 1995).

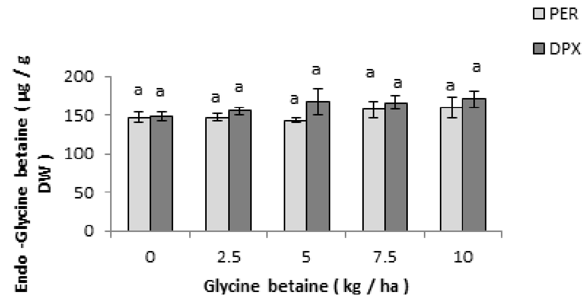

Fig 4. GB content in the leaves of soybean [*Glycine max* (L.) Merr.] cv. Pershing and DPX in leaves of seeding stage in saline soil stress after exogenous application of GB. Values represent mean-SE of four replications plants.

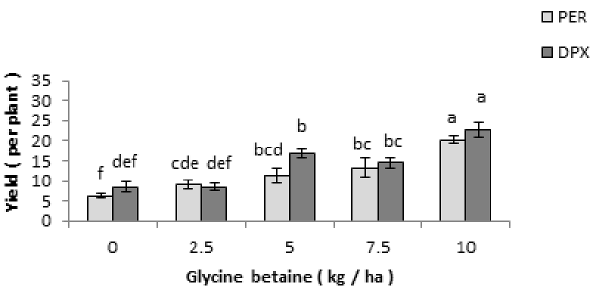

Fig 5. Yield in soybean [*Glycine max* (L.) Merr.] cv. Pershing and DPX in saline soil stress after exogenous application of GB. Values represent mean-SE of four replications plants.

Materials and methods

Plant materials

Seeds of soybean [*Glycine max* (L.) Merr.] cv. Pershing and DPX were prepared from the Agricultural Research Centre, Gorgan, Golestan, Iran.

Soil characteristics and experimental design

Field studies were conducted during the crop season 2009-2010 at the experimental farm of Nokandeh city, Iran. Soybean was planted in spring season on a clay loam saline soil (EC =11.1 and pH=7.6 and Cl^-, Na^+ was 4.2 and 12.5 m.e/lit, respectively). The plot size was 6 m^2 (2×3) and planting density was approximately in rows of 50 cm apart. The experiment was arranged in a Randomized Complete Block Design (RCBD) with four replicates consisted of an untreated control and glycine betaine sprayed at the rates of 2.5, 5, 7.5 and 10 kg ha^{-1}.

GB application and measurement

The foliar application of five Exo-GB treatments was applied at two physiological stages. (1) At six-foliar stage and close to one week before the first flowering. The doses of glycine betaine were put in main plots. The crop was sprayed with a sprayer base on kg GB and 250 L ha^{-1}.

Endo-GB measurement was performed ten days after the treatment in vegetative (plants of ten foliar stage) and reproductive stages (leaves of seeding stage), respectively.

GB extraction and quantification

Estimation of Endo-GB was done on dried leaf powder. Powdered plant material (0.5 g) was mechanically shaken with 20 ml of de-ionized water for 24 h at 25°C. The samples were filtered and then thawed extracts were diluted as 1:1 with 2N sulfuric acid. Aliquot (0.5 ml) was measured in test tube and cooled in ice water for 1 h. Cold potassium iodide-iodine reagent (0.2 ml) was added and the mixture was gently mixed with vortex mixture then centrifuged at 10000 g for 15 min at 8°C. The periodite crystals were dissolved in 9 ml of 1,2-dichloro ethane and after 2.0-2.5 h the absorbance was measured at 365 nm with UV-visible spectrophotometer (Based on Sairam et al., 2000).

Determination of sodium and chloride

Leaf samples were dried in an oven at 105°C for 15 min and then at 80°C to a constant weight. Then, approximately 50 mg of dried leaves was burnt into ashes in an oven at 650°C. The ashes were dissolved in HNO_3 solution and diluted with distilled water to 250 ml. The sodium was estimated by flame-photometer (Tandon, 1995).

Yield attributes

Yield attributes such as grain yield per plant and 500-grain weight were recorded at maturity. Soybean yield estimate equation: (pods per plant) × (seeds per pod) × (weight of 1000 grain) = (per plant)

Statistical analysis

All data obtained were subjected to one-way analysis of variance (ANOVA) and the mean differences were compared by a Lowest Standard Error of mean test (SE). Each data point were a mean of four replicates and comparisons with p values 0.05 were considered significantly different. Statistical analyses of the data were made with SPSS for Windows: Release 19.0-standard version.

Conclusions

Field experiments conducted in saline soils have indicated that the crop stability and yield was often increased due to foliar applications of GB when sprayed during the stress. The physiological basis of yield increases is still under investigation but there are implications that GB might affect on vegetative (number of lateral branch and pod per plant) and reproductive (weight of 1000 grain) stages in soybean plants.

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